COMPARISON OF CIRCADIAN CHANGES IN THE RETINAS OF MIGRATING AND NON-MIGRATING BLACKCAPS, *Sylvia atricapilla*

A Senior Scholars Thesis

by

ROSS SHOCKLEY

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Comparison of Circadian Changes in the Retinas of Migrating and Non-migrating Blackcaps, *Sylvia atricapilla* (April 2006)

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The retinas of birds and other vertebrates undergo morphological and physiological changes throughout the day, which help regulate daily changes in visual sensitivity. One particular change is the contraction and elongation of photoreceptor outer segments, which is regulated by an endogenous circadian clock. Normally, Blackcaps (*Sylvia atricapilla)* are diurnal, but they migrate at night. In the current experiment, outer segment lengths of migrating and non-migrating Blackcaps were measured throughout the day and analyzed, and they show a trend toward circadian rhythmicity across the day. There also appears to be an advance in the phase of the rhythm in retinomotor movements when birds are migrating. These results indicate that circadian-controlled retinomotor movements could be modifications within the retina to accommodate the nocturnal lifestyle of migratory Blackcaps. We hypothesize that a change in retinal

physiology and morphology may allow the formation of a migratory “night-vision” to accommodate the migrating Blackcap’s nocturnal lifestyle.

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INTRODUCTION

The Blackcap, *Sylvia atricapilla*, resides in northern Europe and migrates south in mid-October for the winter. In early April they will fly back north, repeating the cycle. It is well known that the migratory patterns of passerine birds are rhythmic processes, occurring only during certain seasons and at specific times of the day. As such, they have been determined to be under the direct control of endogenous circannual and circadian clocks in addition to being modified and limited by photoperiod. Caged passerine birds (such as the Blackcap) are diurnal during the summer and winter months, however when migrating (autumn and spring), they demonstrate high levels of nocturnal activity, commonly called Zugunruhe (Gwinner, 1996), or migratory restlessness. The birds express their internal migratory drive by flapping their wings and jumping up and down on their perches, all while facing the direction in which they wish to fly (Bartell et al., 2005). This night activity in caged birds occurs even if no external seasonal cues are present, indicating that the circannual timing system is what triggers this change to nocturnal living (Gwinner, 1996).

The switch to nocturnal living is also accompanied by a reduction in melatonin levels, however the exact cause for the significant decrease in melatonin in the body is not known. It is possible that the onset of Zugunruhe is preceded by the drop in melatonin levels. On the other hand, the reduction could be the result of an "increase in the nocturnal light intensity perceived by the birds", which could then lead to a

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This thesis follows the style of *The Journal of Neuroscience.*

high level of night activity (Gwinner, 1996). In either case, it appears likely that the drop in melatonin levels could affect retinal sensitivity, since blood-borne melatonin levels do affect visual sensitivity in other species of birds (Lu et al., 1995).

The magnetic field of the Earth creates a consistent source of directional information which can be used for navigation by migrating birds. This results in electron spin conformational changes as the bird enters different electromagnetic fields. This process implicates the use of retinal Cryptochrome1 and Cryptochrome2 in navigation, because these molecules have been shown to relay magnetic information to the avian brain (Moller et al., 2004). Cry1 and Cry2 have both been shown to exist in the retinas of songbirds (Mouritson, 2004), including species in the genus *Sylvia*, so it is likely they exist in the Blackcap retina.

Photoreceptor outer segments (see Figure 1) of many vertebrates contract and elongate under the direct control of their circadian systems and light. It is thought that the pigmented epithelium aggregates during the night to increase the ability to capture light, and the outer segments elongate during the day to enhance visual acuity (Douglas, 1981). These observations suggest that photoreceptor outer segment movements could help regulate visual sensitivity in Blackcaps too.

We are interested in investigating whether or not differences in retinomotor movements between migrating and non-migrating Blackcaps exist and, if so, what affect these changes have on visual acuity. Additionally, we are interested in the differences in the spatial and temporal regulation of Cry1 and Cry2 proteins between migrating and non-

migrating Blackcaps. The presence of changes in retinomotor movements and cryptochrome expression could be indicative of whether or not the retinas are being "primed" for nocturnal activity when they migrate.

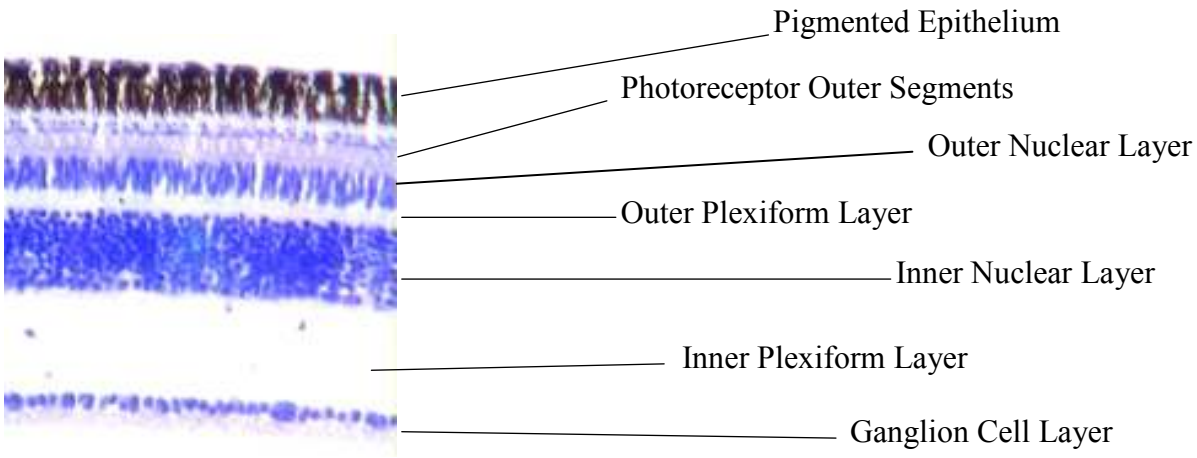

Figure 1. Overview of Retinal Morphology. In this study, retinomotor movement in the outer segment layer was measured.

MATERIAL AND METHODS

Animals

Male Blackcaps (*Sylvia atricapilla)* were caught in Tovetorp, Sweden, at the University of Stockholm Zoological field station, with permittage from the Swedish Environmental Protection Agency. They were transported to the Max Planck Institute for Ornithology at Andechs, Germany. Birds were kept individually in aviaries on 12.75:11.25 LD to allow for the expression of a natural circannual rhythm in migrating behaviors. Blackcaps were provided with water, mealworms, and a mixture of eggs, beef hearts, and commercial food for insectivorous birds.

Histology

At each timepoint (N=3 for ZT 0, 3, 6, 9, 12, 15, 18, 21), the birds were transcardially perfused with heparinized saline followed by perfusion with 4% paraformaldehyde, postfixed with 4% paraformaldehyde, dehydrated in an ethanol series, and imbedded in paraffin. Imbedded eyes were attached to wooden blocks and cut into 7µm sections with a microtome. Sections were float mounted and dried onto slides, deparrafinized, rehydrated, and stained with cresyl violet to visualize Nissl substance. Subsequently the slides were dehydrated in an ethanol series, cleared in Xylene, and coverslipped with Permount.

Image Acquisition

A digital camera connected to an Olympus microscope was used to acquire retinal images, which were subsequently saved to disk. Photoreceptor lengths were measured using ImageJ software (freeware, NIH).

RESULTS

Figure 2a results

Figure 2a displays the mean photoreceptor outer segment lengths from migrating birds throughout the day. Zeitgeber Time (ZT) is shown on the x-axis. ZT 0 is when lights came on.

The peak outer segment occurred at ZT 15 and the greatest length is 60µm. The shortest length is approximately 35µm, and occurs at ZT 12. Thus an overall amplitude of approximately 25µm was observed.

An ANOVA test was performed on the data, and a p-value of 0.12 was obtained. Although this p-value indicates insignificance, the data has a clear trend towards rhythmicity. Statistical analyses, such as cosinor analysis, could be performed.

Figure 2b results

Figure 2b shows the mean photoreceptor outer segment lengths from non-migrating birds across time. Peak length occurs at ZT 21, while the shortest outer segment length was at ZT 12, yielding an amplitude of approximately 20µm.

An ANOVA test was performed on these data and the p-value showed the data to be insignificant. Additional statistical analysis will be performed in an attempt to resolve the problem of too small numbers (N=3).

Figure 2a. Photoreceptor Length vs. ZT time in Migratory Birds

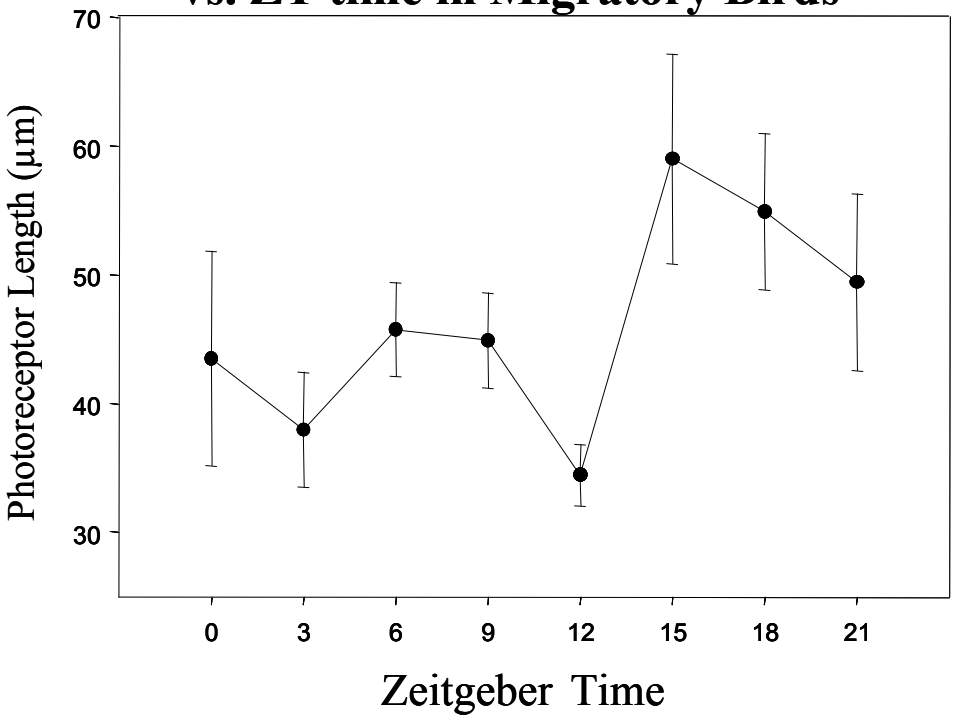

Figure 2b. Photoreceptor Length vs. ZT time in Non-migratory Birds

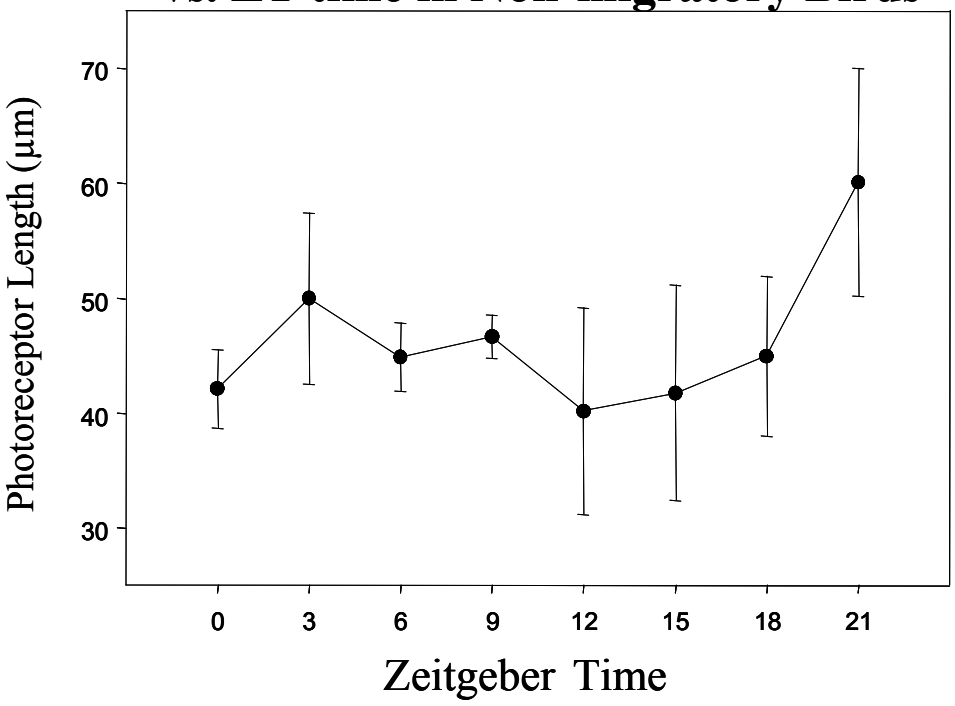

Figure 2. Mean photoreceptor outersegment lengths from the retinas of (a) migrating and (b) non-migrating Blackcaps (N=3 at each timepoint).

CONCLUSION

It is well established that the spatial and temporal migratory patterns of birds are controlled by circannual processes. As birds prepare to migrate, many morphological and physiological changes occur in their bodies. Until now, it had not been investigated whether changes in the visual system (including photoreceptor outer segment length) occur in respect with migration.

Data in graphs 2a and 2b both show a clear trend toward circadian rhythmicity. Unfortunately, the data were shown to be insignificant, likely due to the low number of birds (3) used per timepoint. To increase the experimental power, a continuing study using more birds at each timepoint could be conducted. Using the data collected from the current animals, other statistical tests could be performed that (a) compare the data between each group to determine if there is a correlation between them and (b) determine if the data are rhythmic using alternate non-linear statistical methods. Regardless of the results with ANOVA, it is still worthwhile to compare the data from two graphs, pointing out similarities and differences, and the potential significance of those findings. First of all, the amplitudes of retinomotor movements across the day were approximately 20-25μm for both migrating and non-migrating birds. Similar maximum and minimum photoreceptor outer segment lengths are also observed, however, these maximum and minimum values do not appear at the same times of day under the different migratory condition. Migrating birds have a 6 hour phase advance in peak maximum photoreceptor outer segment length relative to the non-migrating birds.

Also, the duration of peak expression is much longer (9 hours vs. 3 hours) when birds are migrating.

These differences could be the result of modifications within the retina to accommodate the nocturnal lifestyle migratory birds undertake. A series of microphotographs representing the different timepoints is shown in Figure 3.

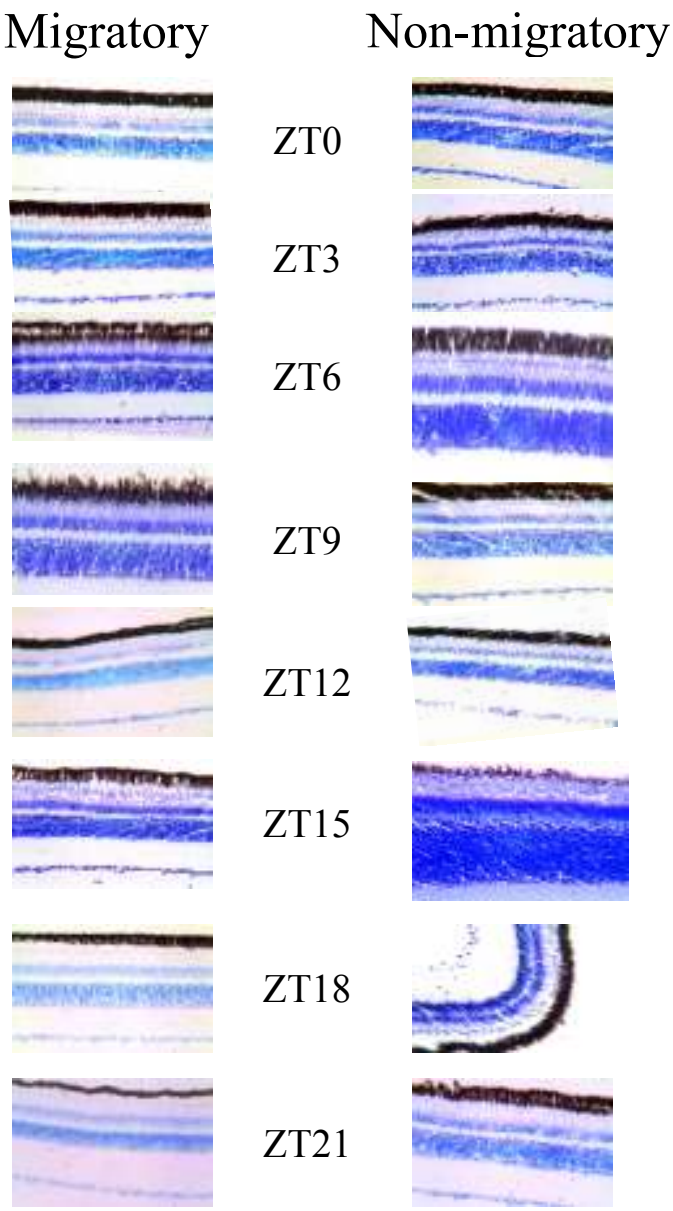

Figure 3. A sequence of representative microphotographs. These microphotographs are from different times of day under both migratory and non-migratory conditions

The changes in retinal morphology we observed could have several widespread implications. Most evident amongst these are that birds could potentially have better eyesight at night while they are migrating, as opposed to when they are not migrating. Lu (1995) has already concluded that a drop in melatonin levels could lead to an increase in visual sensitivity. Since previous studies have shown that when a bird begins to migrate its levels of blood-borne melatonin declines, the implication of pineal melatonin on visual sensitivity in migrating Blackcaps is clear. Future studies specifically examining electrical activity in migrating birds would be fruitful.

Secondly, changes in photoreceptor outer segment length in the retina could lead to modified levels of Cry1 and Cry2 expression. The magnetic field of the Earth is a source of directional information that migratory birds can use. It has been suggested that the direction of the Earth's magnetic field is picked up by radical processes in specialized photoreceptors (Moller, 2004). It is already known that the eye is the location of this magnetoreception (Wiltschko, 2002) and that cells in the retina that contain Cry1 and Cry2 are involved in sending geomagnetic information to the brain at night, the time when birds orient themselves on their journeys (Mouritson, 2004). Furthermore, it has been suggested that cryptochromes play a direct role in mediating magnetic compass information (Moller, 2004) and that garden warblers have a much greater level of expression of Cry1 than non-migrating passerines. This difference is especially distinct in the ganglion cells that are known to extend to the region of the brain that has high levels of magnetically sensitive neurons, the basal optic root

(Mouritson, 2004). These results imply that migrating and non-migrating birds potentially have different degrees of magnetoreception.

The avian circadian system in birds is composed of at least three separate clocks that occur in the retina, the pineal gland, and the hypothalamus (Kumar, 2004). Up until now, we have only discussed the retinal clock; however, the changes in photoreceptor length experienced by migratory birds could in turn affect the phase relationship between all three of the bird's internal oscillators, in particular, between the suprachiasmatic nucleus (located within the hypothalamus) and the pineal gland. For example, an increase in light as perceived by the retina could lead to a phase shift in the suprachiasmatic nucleus (SCN). Increased light sensitivity has also been shown to create changes in phase between the SCN and the pineal gland in several species (Gwinner, 1989).

Continuing studies could investigate what neurochemical changes take place in the retina across the day during migration. An example of such a study could involve visualizing melatonin receptor immunoreactivity. An increase in melatonin receptor number in the nocturnal, migrating birds would correspond to a decrease in melatonin levels. These immunohistochemical studies will be the focus of future investigation by me next year.

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